Plant Archives Vol. 20, Supplement 1, 2020 pp. 3041-3049 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

WHEAT AND PEA SEEDLINGS AS PRODUCERS OF BIOLOGICALLY ACTIVE COMPOUNDS

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Abstract

Species-specific (wheat, pea) patterns of root exometabolite excretion were observed. Thus, wheat seedling exudates have a high content of carbohydrates (50-67%), while the major component of pea seedling exudates is proteins (80-85%). There was nearly a liner increase in the exometabolite content in wheat exudates from Day 1 through Day 3, while no or little increase in exometabolites was observed only in Day 3 pea exudates. Pre-sowing treatment of pea seeds using Methods 1 and 2 did not affect the root growth rate, while using Method 3, a two-fold increase in the root growth rate from Day 1 to Day 3 was observed compared with the control. An increase in the pea root growth rate was associated with a 34% increase in root excretion in Day 3 seedlings, however, the ratio of proteins, free amino acids and carbohydrates remained unchanged compared to the control. Pea but not wheat exometabolites supported *B. cereus* culture, suggesting that the differences in the root exudate composition affect the composition of the root rhizosphere microbiota. With addition of Day 1 pea root exudates to the wheat seedling culture medium, root growth rate increased by 58 % compared with the control, whereas when Day 1 wheat root exudates were added to the pea seedling culture medium, there was only a 31% increase in the growth rate compared with the control. This paper discusses potential biotechnological applications of root exometabolites as biologically active substances with pronounced biological activity.

Keywords: Pea and wheat root exometabolites, root exudates, root rhizosphere, microbiota, biologically active compounds.

Introduction

It is known that plant roots excrete a large number of various exometabolites into the environment (Carvalhais *et al*., 2015). Root exudates perform various functions, such as regulation of root micrbiome composition and concentration of microorganisms (Huang *et al*., 2014; Hugoni *et al*., 2018) which provide the plant with nutrients; protect the plant from pathogens (Keiluweit *et al*., 2015) and provide allelopathic relationships in plant communities (Bertin *et al*., 2003; Bouhaouel *et al*., 2015; Bais *et al.,* 2006). It is known that the intensity of root excretion and the root exudate composition may vary depending on the plant species, conditions of growth, microbiota profile and the stage of plant ontogenesis. Understanding the characteristics of the root excretory system and species-specific aspects of its functioning is highly important to solve problems of plant resistance and development.

Along with this, root exometabolites are promising targets for biotechnology due to their wide spectrum of biological activity (Kamath *et al.,* 2004; Zhang *et al*., 2015; Cai *et al.,* 2012). It was shown that the effects of wheat root exudates obtained during early culture (Day 1-3 of growth) were not limited to only plants, but also favored regeneration of liver cells (Kuznetsova *et al*., 2019). In this regard, investigation of qualitative and quantitative characteristics of root exudates in different plant species is important both from fundamental and practical points of view.

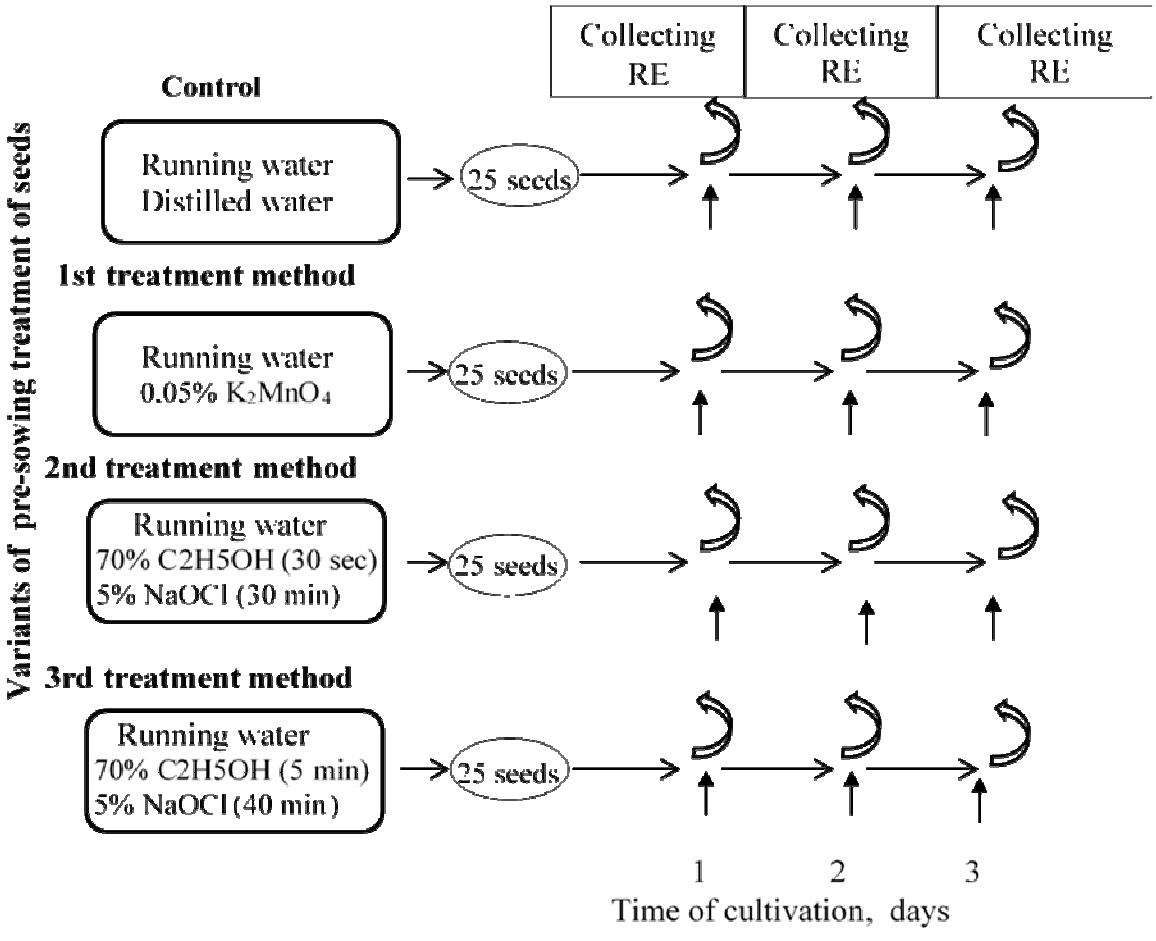
It was previously shown that inhibition of root growth in Day 1-Day 3 wheat seedlings was associated with an increase in the root excretory activity (Kuznetsova, 2008; Bozhkov *et al*., 2009; Bozhkov *et al*., 2013). Since water culture requires pre-sawing treatment of seeds (hereinafter – "pre-treatment") in order to remove any accompanying microflora, the objective of this study was to investigate the influence of different methods of pre-treatment of pea seeds and the effect of such pre-treatment on the root growth rate and qualitative and quantitative characteristics of root excretion, and to evaluate biological activity of root exudates in the model system.

Soil microorganisms play an important role in the formation of the rhizosphere. Using root exudates as a substrate, they are involved in plant growth and development (Bais *et al.,* 2006; Huang X-F, 2014). A typical member of the rhizosphere microbiota is *Bacillus cereus*.

Therefore, this study was focused on investigation of different methods of pre-treatment of pea seeds and their effect on the root growth rate; qualitative and quantitative comparison of root exometabolite profiles in Day 1 through Day 3 water-cultured wheat and pea seedlings; the relationship between the intensity of root growth and the excretory activity of pea roots during Day 1 to Day 3 of growth following pre-treatment using different methods; the effect of pea root exudates on the growth rate of wheat roots and *vice versa*: the effect of wheat root exudates on the growth of pea roots; and, finally, the ability of wheat root exudates to support the growth of *B. cereus*, a typical member of the rhizosphere microbiota.

Materials and Methods

Seeds of pea (*Pisum sativum*) and wheat (*Triticum aestivum*) were washed in running tap water for 3 minutes and then rinsed three times with sterile distilled water. After this washing, seeds were divided into 4 groups. First group was not further treated after washing and used as the Control (Fig. 1)



↑ **Adding a new portion of sterile distilled water to Petri dishes**

Fig. 1 Scheme of seed pre-treatment, culture and timing of root exudate (RE) collection

Next group was then washed in 0.05% potassium permanganate solution (K_2MnO_4), and then rinsed with sterile distilled water. This was Method 1 of seed pre-treatment (Fig. 1).

Another part of washed pea seeds was soaked in 70% ethanol for 30 seconds, then rinsed washed with sterile distilled water, then soaked in 5% sodium hypochlorite for 30 minutes and again rinsed with sterile distilled water. This was Method 2 of seed pre-treatment (Fig. 1).

The third part of washed pea seeds was soaked in 70% ethanol for 5 minutes, rinsed with sterile distilled water and soaked again in 5% sodium hypochlorite for 40 minutes and rinsed with sterile distilled water. This was Method 3 of seed pre-treatment (Fig. 1).

After pre-treatment, all groups of pea seeds were soaked in sterile distilled water for 24 hours at 26°C. After 24 hours of soaking, sprouted seeds from each treatment group were placed into 9.5 mm Petri dishes with 25 seeds/dish, so that only a small part of each seed was immersed in water. Three Petri dishes were made for each group meaning 3 technical replicates during each biological replicate. For this, 10 ml of sterile distilled water was added to the Petri dishes (Fig. 1). In order to exclude the risk of extraction of substances from the seeds, a test was carried out. For this, the medium where non-germinated seeds were cultured for 24 h was assayed and no proteins and carbohydrates were detected there. This confirmed that excretion of exometabolites from seeds themselves would not affect root activity. Therefore, medium would only contain substances that are specifically excreted by the roots of germinating seeds, i.e. root exometabolites.

Then dishes with wheat and pea seedlings were placed in a chamber with continuous light at 25°C for 1, 2, and 3 days.

Culture conditions for wheat and pea seeds

Pre-treated using different methods and cultured in 9.5 cm Petri dishes seeds were analyzed and the number of non-germinated seeds was counted on Day 1, Day 2 and Day 3, to calculate the seed germination percentage.

In the next experiment, to obtain root exometabolites we selected germinated seeds and placed them in Petri dishes with 25 seeds per dish, added 10 ml of sterile distilled water and cultivated them at 25°C and 4 kLx light intensity. After 24 hours, aqueous solution of root exometabolites was collected under sterile conditions (this was the solution of Day 1 seedling root exometabolites) (Fig. 1). To the remaining in the same Petri dishes seedlings, another 10 ml of distilled sterile water were added, and seedlings were cultured under the same conditions for another 24 hours. After that, aqueous solution of root exometabolites was collected the same way - the solution of Day 2 seedling root exometabolites (Fig. 1). The same was done on Day 3 of seedling growth (Fig. 1). Therefore, a "flow-through" mode was used for collection of root exometabolites.

Total proteins, total carbohydrates and free amino acids were measured in aqueous Day 1, Day 2, and Day 3 solutions of root exometabolites, and the total amount of these compounds per 100 roots was calculated.

Collection and border cell count

Roots of wheat and pea seedlings were fixed in 2% glutaraldehyde and stained with 0.06% Trypan Blue. Then, apical parts of the roots were placed on the object slide in a drop of water and covered with a cover glass. The number of border cells in the apex was counted under a light microscope (LOMO, Russia) and calculated per root (Bozhkov *et al*., 2013).

For border cell preparative isolation, 0.5 ml distilled water was added to a compartment of a plastic culture plate fixed on a magnetic stirrer. The 1.5–2.0 cm long apical root zone (without detaching the root from the caryopsis) was plunged into actively stirring water for 1–2 min in order to remove the apex gel sheath with border cells. In one compartment, 20 to 50 roots were treated. Washing medium was transferred from a plate compartment into test tubes and centrifuged at 3000 g for 15 min in order to sediment border cells. The pellet containing border cells was fixed with 2% glutaraldehyde and stained with 0.06% Trypan Blue. The number of border cells removed by preparative method was counted in a Goryaev hemocytometer and calculated as a number of cells per root (Bozhkov *et al*., 2013).

Total protein content, total carbohydrates and amino acids in root exudates

The classical Lowry method was applied to measure protein content of root exudates (Lowry, 1957; Kuznetsova, 2008). The carbohydrate content of root exudates was measured as described in (Masuko, 2005; Kuznetsova, 2008). The content of free amino acids in exudates was measured according to the ninhydrin method described previously (Kuznetsova, 2008; Azeez *et al*., 2018).

Preparation of nutrient medium from Day 2 wheat and pea root exudates for a pure culture of *B. cereus*

In order to determine whether root exometabolites can support microorganism (*B. cereus*) growth, agar-based culture media were prepared from wheat and pea root exudates and the growth rate of *B. cereus* in these media was determined. As a control, standard meat-peptone agar was used (3% agar).

To prepare the wheat and pea root exudate-based agar medium, root exudates of wheat and pea were collected on Day 2 of seedlings' growth. Root exudates from each plant species were concentrated in a vacuum evaporator twice and added 3% agar-agar.

Using wheat and pea root exudates as a germination media for pea and wheat, respectively

One-day old wheat and pea seedlings were washed with distilled water to remove their own exudates. To determine the mutual influence of pea root exudates on wheat root growth and *vice versa*, - of wheat root exudates on pea root growth, the following scheme was used.

At the first stage, aqueous solutions of one-day-old wheat and pea root exudates were obtained and processed as previously described. The composition of the root exudates was previously determined.

Then, processed wheat root exudates were introduced into Petri dishes with pre-washed one-day old pea seedlings. Accordingly, processed pea root exudates were added to Petri dishes with pre-washed one-day old wheat seedlings. This way, a reciprocal exchange of root exometabolites was performed.

At the following stage, seeds of peas and wheat were cultured at 24 °C with corresponding root exudates added into culture medium. The length of germinated roots was measured after 24 and 48 hours of culture. Based on this parameter, the effect of pea root exometabolites on the wheat growth and the effect of wheat exometabolites on pea growth was determined.

Statistical analysis

All experiments were carried out in 3 technical replicates (1 plate with 25 seeds each) and 3 biological replicates. Therefore, 225 seeds of pea or wheat were analyzed in each treatment group. For each parameter studied, the mean and the standard error of the mean were calculated. The differences between samples were analyzed using the non-parametric Mann-Whitney U-test (Mann and Whitney, 1947). Differences were considered statistically significant at $P<0.05$.

Results

The effect of pre-treatment of pea seeds on germination and growth rate of roots

After one day of soaking, 16% of the seeds remained non-germinated in the control group. After two days, this value decreased to 8% and remained unchanged on Day 3 of growth (Fig. 2A). None of the three methods of seed pre-treatment had any significant effect on germination of pea seeds (Fig. 2A). Consequently, such seed treatments did not have any toxic effects on seeds.

After one day of growth, the mean length of pea roots in the control group was 0.58 cm, and by the end of Day 2 it reached 1.37 cm, suggesting the specific growth rate during Day 2 was higher than during Day 1 (Fig. 2B, C). On Day 3 of growth, the specific growth rate decreased compared with Day 2 (Fig. 2C). Therefore, in the control, the growth rate of pea roots from Day 1 through Day 3 was nonlinear.

Pre-treatment of pea seeds using Methods 1 and 2 did not have any significant effect on the root growth rate and the patterns of their specific growth rates (Fig. 2). However, pre-treatment of pea seeds using Method 3 was accompanied by a significant increase in the growth rate of pea roots: after pre-treatment using Method 3, the length of pea roots on Day 1 of growth reached 1.2 cm, thereby twice exceeding the value in the control group (Fig. 2B). At the same time, the pattern of the specific growth rate in this group did not differ from that in the control (Fig. 2C).

At the next stage, the relationship between the growth rate of pea roots and the rate of root exometabolite excretion into medium was determined. We found that pea root exometabolites contained 80-85% of proteins, while in wheat the main proportion of exometabolites made carbohydrates (50-67%) (Table 1).

A

Day of growth	Methods of pre-sowing treatment			
	Without treatment	Method 1method	Method 2	Method 3
1	16.0±0.8	16.0±1.0	12.0±0.8	12.0±0.5
2	8.0±0.5	12.0±0.6	8.0±0.2	12.0±0.8
3	8.0±0.3	8.0±0.5	8.0±0.5	12.0±0.5

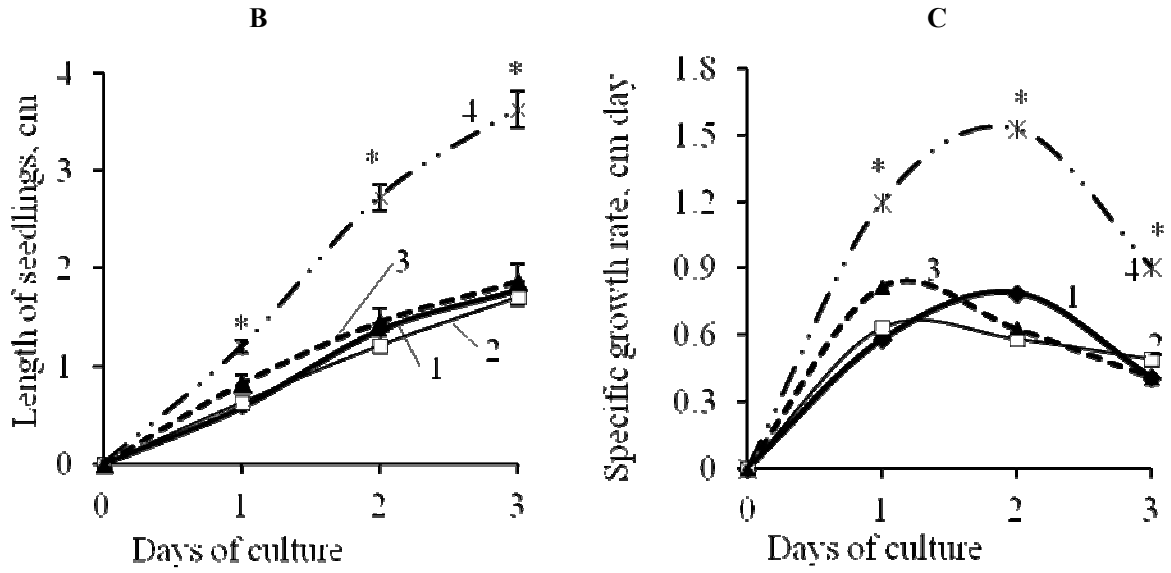


Fig. 2 : Proportion of non-germinated pea seeds on Day 1, Day 2 and Day 3 in % (A), the length of seedlings on Day 1, Day 2 and Day 3 (B) and the specific growth rate of pea root seedlings (C) in the control (without pre-sowing treatment - 1) and following pre-treatment using different methods: 2 – Method 1 (0.05% potassium permanganate solution); 3 - Method 2 (30 seconds in 70% ethanol and 30 minutes in 5% sodium hypochlorite); 4 – Method 3 (5 minutes in 70% ethanol and 40 minutes in 5% sodium hypochlorite)
* - indicates a significant difference ($P < 0.05$) compared with the control (untreated seeds)

Table 1: Free amino acids, proteins and carbohydrates in wheat and pea root exudates and the ratio of these compounds in pea vs. in wheat exudates

Content of root exometabolites	Day of growth	Wheat	Pea	Pea to wheat ratio + - increase - - decrease
protein, µg 100 seeds^{-1}	1	338.5±40.8	4961.7±128.4	+ 14.6 times
	2	693.8±101.4	4716.3±233.3	+ 6.8 times
	3	1456.3±158.4	5654.3±381.4	+ 3.9 times
amino acids, µg 100 seeds^{-1}	1	130.0±14.7	274.9±62.9	+ 2.1 times
	2	702.4±55.2	337.0±68.5	- 2.1 times
	3	1112.2±64.4	465.5±40.8	- 2.4 times
carbohydrates µg 100 seeds^{-1}	1	949.7±106.2	597.1±32.2	- 1.6 times
	2	1342.8±98.2	718.2±35.6	- 1.9 times
	3	3311.5±318.3	975.9±58.8	- 3.4 times

Further, the patterns of excretory activity of pea and wheat roots differed from Day 1 through Day 3 of growth. The content of proteins, free amino acids and carbohydrates in wheat exudates increased from Day 1 through Day 3 almost linearly, while in pea exudates there were little or no changes in the content of these groups of exometabolites (Table 1). The differences in the patterns of wheat and pea root excretory activity are further confirmed by the changes in ratios of proteins, amino acid and carbohydrate content in root exudates on Day 1, Day 2 and Day 3 of seedlings growth in wheat and pea. Protein content of Day 1 pea root exudates was 14 times higher compared with wheat exudates, while Day 3-old pea seedlings excreted only 3.9 times more protein compared with wheat (Table 1).

Therefore, wheat and pea root exudates differed in composition and patterns of exometabolite excretion into water medium. Since excretion of proteins and amino acids is of a great interest in terms of practical applications, at the following stage we investigated the relationship between pea seedlings' root growth intensity, which varied depending on the method of seed pre-treatment, and their excretion of proteins, amino acids and carbohydrates.

The effect of seed pre-treatment on the quantitative content and qualitative composition of exometabolites in pea seedlings from Day 1 through Day 3 of growth

As was shown above, protein content of pea root exudates made over 80% of all compounds, and this amount changed little from Day 1 to Day 3 of culture. Pre-treatment of seeds using Methods 1 and 2 did not change protein content (Table 2). However, after seed pre-treatment using Method 3, the content of proteins in root exudates increased by about 20%, 14% and 33% on Day 1, Day 2 and Day 3 of growth, respectively, compared with the control (Table 2). Therefore, pre-treatment of pea seeds using Method 3 resulted in an increase in the growth rate of seedlings and an increase in the protein content of root exudates.

Table 2: Total protein, free amino acids and carbohydrates in root exudates of pea seedlings on Days 1, 2 and 3 after different pre-sowing treatment

Content of root exometabolites	Day of growth	Control	Pre-treatment method		
			Method 1	Method 2	Method 3
protein, mg 100 seeds^{-1}	1	4.9±0.1	4.7±0.2	4.6±0.2	5.5±0.2
	2	4.7±0.2	4.8±0.2	4.7±0.2	5.4±0.1
	3	5.6±0.3	5.5±0.4	5.5±0.4	7.5±0.4
amino acids, mg 100 seeds^{-1}	1	0.27±0.06	0.30±0.05	0.32±0.08	0.39±0.08
	2	0.33±0.06	0.23±0.03	0.31±0.04	0.24±0.05
	3	0.46±0.04	0.47±0.03	0.46±0.02	0.56±0.02
carbohydrates, mg 100 seeds^{-1}	1	0.59±0.03	0.61±0.04	0.77±0.03	0.78±0.06
	2	0.72±0.03	0.71±0.03	0.84±0.03	0.77±0.06
	3	0.98±0.06	1.06±0.09	0.96±0.05	1.41±0.08

Patterns of free amino acids content in root exudates were similar to that of protein with a 21% increase in amino acid and a 33% increase in protein content after seed pre-treatment using Method 3, compared with the control (Table 2).

Seed pre-treatment using Methods 1 and 2 did not change the carbohydrate content compared with the control. Neither did it change significantly after pre-treatment using Method 3, except on Day 3, when the carbohydrate content of exudates increased by about 43% compared with the control (Table. 2). Therefore, a 2-fold increase in the growth rate of pea roots on Day 3 observed after pre-treatment using Method 3, was accompanied by an increase in the excretory activity of root exudates compared with the control.

Root border cells are known to play an important role in the intensity and characteristics of the root excretion (Hawes *et al*., 1998; Bozhkov *et al*., 2007). Estimation of the number of border cells in the rhizosphere of wheat and pea roots showed that, Day 1 wheat roots had 80-100 border cells per root, while in pea this number varied from one to several thousand border cells per root (Fig. 3). It should be noted that border cells of wheat and pea roots differ in their size and shape (Fig. 3B).

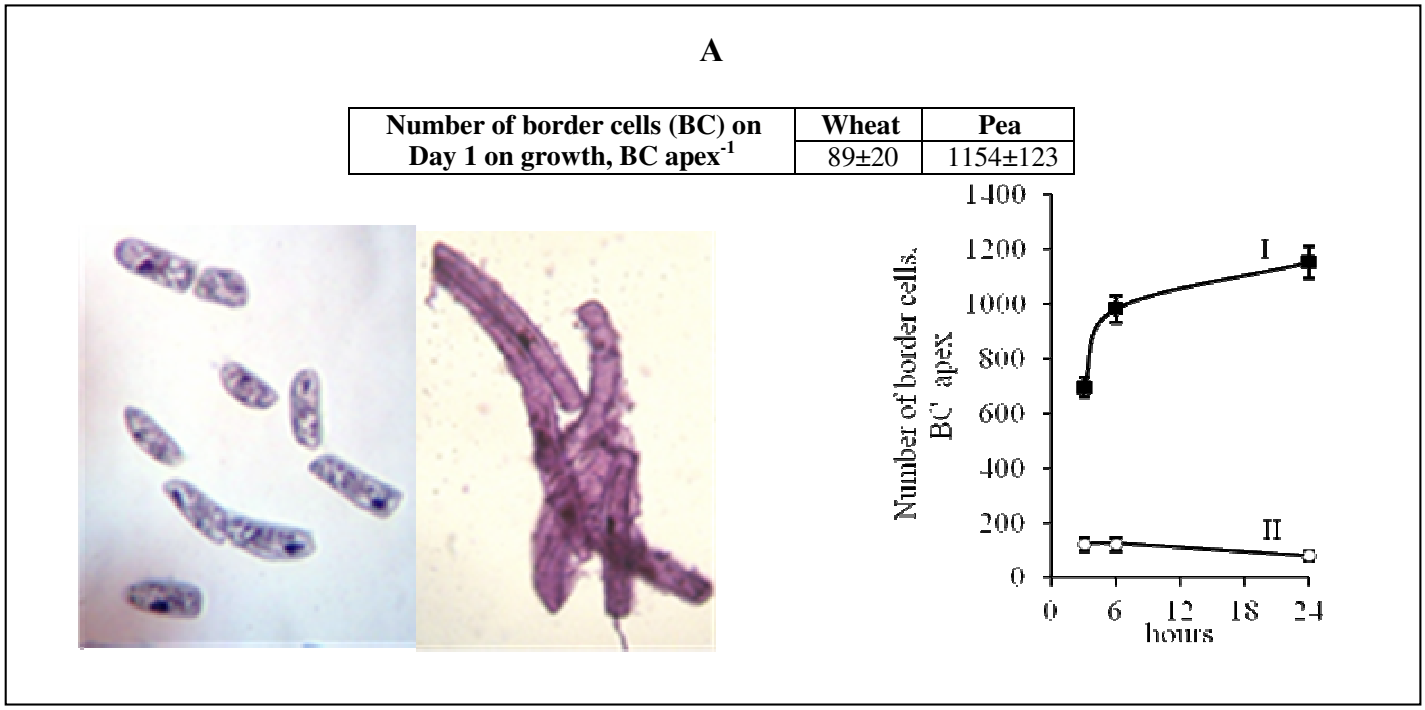


Fig. 3 : Number of border cells in the rhizosphere of wheat and pea roots on Day 1 (A) and morphology of these cells (B). Number of border cells in the rhizosphere of pea (I) and wheat (II) roots after 3, 6 and 24 hours of culture in water medium (C)

In the next series of experiments, the patterns of "transition" of border cells into the rhizosphere of pea and wheat roots were determined. It was observed that already after first 3 hours of culture, pea rhizosphere contained 700 border cells per root, while wheat rhizosphere had only about one hundred cells (Fig. 3C). Such rapid development of the root excretory apparatus can explain the large yield of root exometabolites in Day 1 pea seedlings.

These differences in the quantitative and qualitative characteristics of root exudates may be associated with structural and functional organization of the excretory apparatus of the root in different plant species.

Investigation of biological activity of root exudates of pea and wheat on model systems

In the next series of experiments, the ability exometabolites from wheat and pea root exudates to support *B. cereus* growth was determined. As a control culture medium for *B. cereus*, standard meat-peptone agar (MPA) was used.

When *B. cereus* was cultured with wheat exudates, no growth was observed, suggesting that this bacterium is not able to absorb wheat exometabolites (Fig. 4A). In contrast, when *B. cereus* was cultured with pea exudates, the bacteria formed the same white, rounded colonies as with the control MPA medium (Fig. 4A).

The area of *B. cereus* colonies was measured to evaluate the growth rate of bacterial culture, and no association between the area of colonies and the method of seed pre-treatment was found, but it was 70-80 % less than the area of *B. cereus* colonies cultured in MPA (Fig. 4B), suggesting that root exudates of pea and wheat have different biological activities with respect to soil microorganisms, in particular, *B. cereus*.

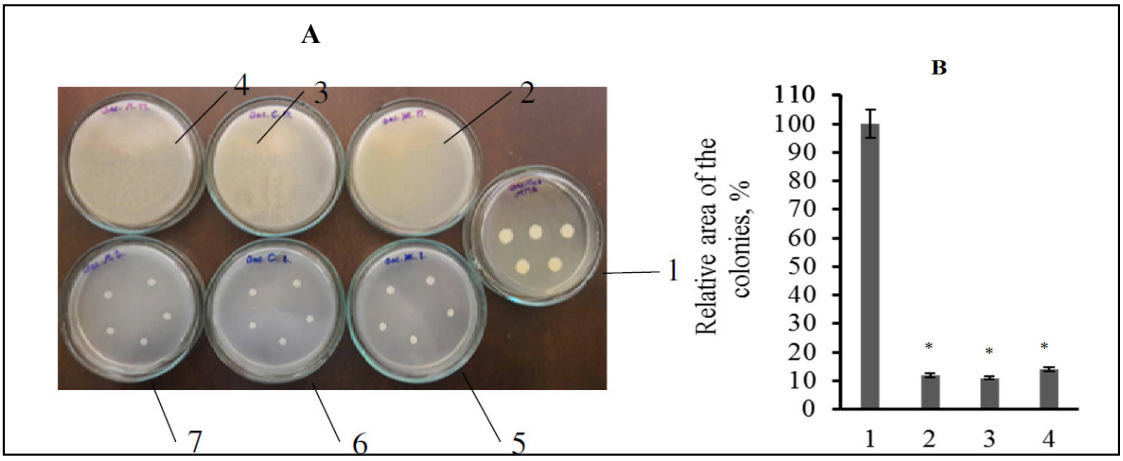


Fig. 4 : Growth of *Bacillus cereus* on agar media (A): meat-peptone agar (1), wheat root exudate-based media (2, 3, 4) obtained from Day 2 seedlings and pea root exudate-based media (5, 6, 7) obtained from Day 2 seedlings. The relative area of the colonies of *Bacillus cereus* (B) grown, respectively, on MPA plate (1), on agar plate with pea root exudates obtained without pre-sowing treatment (2), on agar plate with pea root exudates obtained after pre-treatment using Method 1 (3) and after pre-treatment using Method 2 (4)

* - indicates a significant difference ($P < 0.05$) compared with the MPA

Components of root exudates can provide an allelopathic effect on the growth and development of other plant species. Here we also studied an inter-species reciprocal effect of wheat root exudates on the growth of pea roots, and of root pea exudates on the growth of wheat roots. For this, root exudates obtained from Day 1 pea seedlings were added into Petri dishes with one Day 1 wheat seedlings. It was found that after addition into culture medium, root exudates obtained from Day 1 pea improved wheat root growth by about 31% compared with the control after 24 hours of culture, and by about 58% after 48 hours of culture (Fig. 5). Addition of Day 1 wheat exudates to Petri dishes with Day 1 pea seedling also demonstrated a stimulating effect on root growth, but it was less pronounced compared with pea exudates added to wheat roots (Fig. 5)

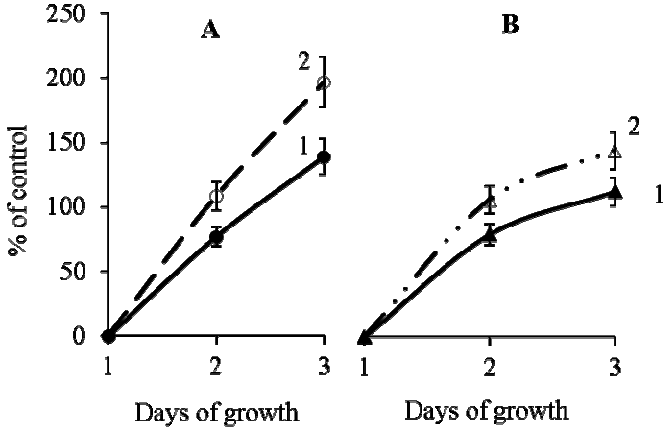


Fig. 5 : The effect of Day 1 pea root exudates on the growth rate of wheat roots (A). The effect of Day 1 wheat root exudates on the growth rate of pea roots (B)

1 - control without root exudates added; 2 - after adding root exudates

Discussion

Summarizing the results of this study, the following main findings can be outlined: (1) the growth rate of pea roots from Day 1 through Day 3 was nonlinear; (2) the patterns of excretion of root exometabolites and their composition differed significantly in wheat and pea at the initial growth stages; (3) pre-treatment of pea seeds with 70% alcohol and sodium hypochlorite stimulated root growth from Day 1 to Day 3 and increased their excretory activity; (4) pea root exometabolites can be absorbed by *B. cereus*, a soil bacterium, while wheat exometabolites cannot make a substrate for growth of these bacteria; (5) pea root exometabolites improved the growth of wheat roots by about 58%, while wheat root exometabolites only improved pea root growth by about 31%.

Speculating about these results, the non-linearity of the root growth rate from Day 1 through Day 3 can be explained by characteristics of the root cell proliferation. It is known, that in the apical meristem of the root cells are aligned in regular rows that are organized in repeating modules (Clowes, 2000; Waisel *et al.,* 2002). Each cell module has its own rhythm of proliferative activity (Barlow, 1987; Кравец, 2012), resulting in a nonlinearity of the root growth rate. Along with this, an expressed daily patterns of the root growth during first days of development is explained by the relatively synchronous passage of cells through the cycle during Days 1 to 3 (Loef, Van Onckelen, 2004). Later, asynchrony of the cell division is observed.

Differences in the patterns of root exometabolite excretion between wheat and pea can be explained by structural differences in organization of the root meristem in pea and wheat. While the root meristem of pea belongs to the so-called 'open' type, and already starting from the first day of root growth a large number of border cells (up to one thousand of BC/apex) and exometabolites are released into the rhizosphere, in wheat, which has a 'closed' type of meristem, the number of border cells released into the rhizosphere, is much lower (up to several hundreds of BC/apex). As a result, the secretory activity of pea roots quickly reaches the plateau, while this is achieved much more slowly in wheat, which excretion reaches the plateau only on Day 3. These features must be taken into account for biotechnological applications using pea and wheat seedlings as producers of biological substances.

The different root exudate composition in pea and wheat strongly suggests that different types of plants can become a source of different composition of biologically active compounds (Kamath *et al.*, 2004; Zhang *et al.,* 2015; Cai *et al.*, 2012).

Differences in biological activity of root exometabolites suggested by the effect of pea root exometabolites on *B. cereus* in this study, further confirm the results of other studies on the effects of wheat root exometabolites on the proliferative activity of liver cells (Kuznetsova *et al.*, 2018) and of a number of pathogenic microorganisms (Kuznetsova, 2008).

Further, an interesting, from research and practical points of view, object is protein-rich pea root exudates. In this study we found that pre-treatment of pea seeds with 70% ethyl alcohol and sodium hypochlorite improved root growth and did not inhibit but increased their excretion.

Root growth can be stimulated by: (1) a specific effect of stimulation by phytohormones or other cytokines (Zhang *et al.*, 2016; Sun *et al.*, 2016); (2) a non-specific effect of low concentrations of toxic compounds, called hormesis. It was shown that low doses of radiation, low concentrations of toxic chemical compounds, in particular heavy metal ions, induce synthesis of specific stress proteins (Emamverdian *et al.*, 2015), cause metabolic changes (Ashrafa and Tang, 2017; Chia, 2015), alter expression of a number of genes (Liu *et al.*, 2019) and in some cases this is accompanied by activation of proliferation. It is known that hormesis is characterized by a U-shaped dose- or time-dependent response to exogenous factors, as was the case here.

It can be assumed that pre-treatment of seeds provided, on the one hand, the "selection" of actively growing plants, and on the other hand, it induced hormesis. Since hormesis is a response to the action of toxic compounds of various nature, it can be assumed that the increase in the excretory activity against the background of a non-specific stimulation of root growth can also be attributed to compensatory hormesis-based adaptation of plants.

In summary, wheat and pea seedlings are promising targets for root biotechnology. Day 1 to Day 3 seedlings can be a source of root exudates of different composition and with different biological activity, and seed pre-sowing treatment can be used for regulation of root growth and excretion.

Conclusions

1. The pre-sowing treatment of pea seeds with 70% ethyl alcohol for 5 minutes and 5% sodium hypochlorite for 40 minutes resulted in a 2-fold increase in the root growth rate. The specific growth rate of pea roots was non-linear but followed a U-shaped curve with a maximum reached on Day 2 of growth.
2. Wheat root exometabolites are mainly represented by carbohydrates (50-67% of the main components), while in peas the predominant group is proteins (80-85% of the main components). Excretion of wheat roots (closed-type of meristem) increased almost linearly from Day 1 through Day 3 of growth, and in pea (open-type of meristem) one day after induction of growth, the excretory activity reached plateau.
3. Pea but not wheat root exometabolites supported growth of soil bacteria *B. cereus*, which suggests that different plant species form specific allelopathic microenvironments.
4. Pea root exometabolites improved the root growth rate of wheat seedings in water culture by 58%, while wheat root exometabolites increased the root growth rate of pea seedings by only 31%.
5. The number of border cells in the rhizosphere of Day 1 wheat roots is 89 cells per apex, and in the rhizosphere of peas 1154 border cells/apex formed during this culture time.

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